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The *in vivo* Increase of Rosette Forming Cells by a Deoxyribonuclease Sensitive Factor in Leucocyte Extract from Animals Serially Administered with Immune RNA

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Summary

It was previously shown that an immune ribonucleic acid (RNA) preparation, extracted from the spleen of fowls immunized with sheep erythrocytes (SRBC), induced rosette forming cells (RFC) with SRBC in chicken blood lymphocytes. The present experiments demonstrated that the induction of the RFC occured when the leucocyte extract from fowls, which had serially received the immune RNA, was administered to other folws. In addition, the deoxyribonuclease (DNase) sensitive substance contained in the extract increased the number of RFC. These results suggest that the DNase sensitive substance plays an important role in maintaining the immunological capacity which can be transferred by the immune RNA.

RNA extracts from lymphoid tissues of animals immunized with various antigens transfer both humoral (1) and cellular (2) immunities to their recipients. The transfer abilities are maintained in the recipient for a long term (3) by the RNA maintained or replicated in the recipient. The author previously reported that an RNA preparation extracted from the spleens of fowls immunized with sheep erythrocytes (SRBC) increases the number of rosette forming lymphocytes (RFC) with SRBC in chicken blood (4) and that the RFC inducing ability can be transferred to the lymphocytes of fowls which serially receive the immune RNA (5). The results suggest that the RNA itself or some substances functionally analogous to RNA were maintained or synthesized in the recipients after a serial administration of the RNA. The present data will show that a deoxyribonuclease-sensitive substance contained in the extract of leucocytes from fowls after the administration of the immune RNA augments the number of RFC.

Materials and methods

Preparation of immune RNA. Fowls (8 to 12 months White Leghorn) were immunized singly with 1–2 ml of a 10% suspension of washed SRBC by intravenous injection. The animals were decapitated at 2 or 3 days after the immunization. RNA was prepared from the spleens by the method described previously (4).

Preparation of the extract of leucocytes after administration of RNA. Four fowls (White Leghorn) weighing about 0.7 kg received intravenously 2.4 mg of the immune RNA per bird, 3 times every 4 days. Four days after the last injection, blood was obtained by cardiac puncture. Leucocytes were separated by a centrifugation on a Ficoll-Hypaque gradient according to the method of Boyum (6). The cells (1.3×10^9) were suspended in 30 ml of 0.15 M NaCl containing 0.02 M sodium citrate, followed by the addition of 3 ml of 10% sodium lauryl sulfate. After a vigorous shaking, 3.3 ml of 10 M NaCl was added. Then, 12 ml of chloroform-butanol (4:1) mixture was added and the suspension was shaken vigorously. The suspension was centrifuged at 13,000 ×g for 10 minutes, then the aqueous phase was collected and added with 90 ml of 95% ethanol. This solution was centrifuged at 13,000 ×g for 10 minutes. The precipitate was dissolved in 7 ml of 1 M NaCl solution. One milliliter of the extract contained 360 μg of DNA, as measured by the diphenylamine method (7). The Extract of the leucocytes from both folws which received and whose which did not receive the nonimmune RNA was prepared in the same manner.

Test of augmentation of RFC by the extract. Extract (0.5 ml each) from the leucocytes of fowls administered respectively with the immune RNA, the nonimmune RNA or not-received any RNA, was injected intravenously into White Lehgorn fowls 5 weeks old. The leucocyte extract of the fowls administered with immune RNA was treated with 1% of deoxyribonuclease 1 (Sigma Chemical Co.) and of ribonuclease A (Sigma Chemical Co.) at 37°C for 30 minutes, respectively. The 0.5 ml of each extract was injected as described above.

In order to assay the RFC, the blood lymphocytes were prepared according to the method of Boyum (6) at 2 days before, and at 3, 8 or 12 days after the injection of the extracts. The number of RFC with SRBC was counted by the method described previously (4).

Results

The increase of RFC induced by the lymphocyte extract in fowls via both immune RNA and nonimmune RNA and those receiving no RNA was examined. Fig. 1 shows an induced increase of RFC at 3 days of injection for fowls which received the lymphocyte extract from fowls to which immune RNA had been administered. Neither the leucocyte extract from fowls administered with non-immune RNA nor that from the non-administrated fowls induced RFC (Fig. 1). These results show that substances contained in the leucocyte extract from fowls

administered with immune RNA augment RFC.

Also, the increase of RFC induced by the lymphocyte extract treated with deoxyribonuclease 1 or ribonuclease was examined. The number of RFC in the recipients of the deoxyribonuclease 1-treated leucocyte extract did not exceed the level before the injection, whereas the number of RFC in the recipients of the ribonuclease A-treated leucocyte extract was almost the same number as that in the recipients of the ribonuclease A-not treated extract.

These results show that deoxyribonuclease sensitive substances contained in the extract augment RFC.

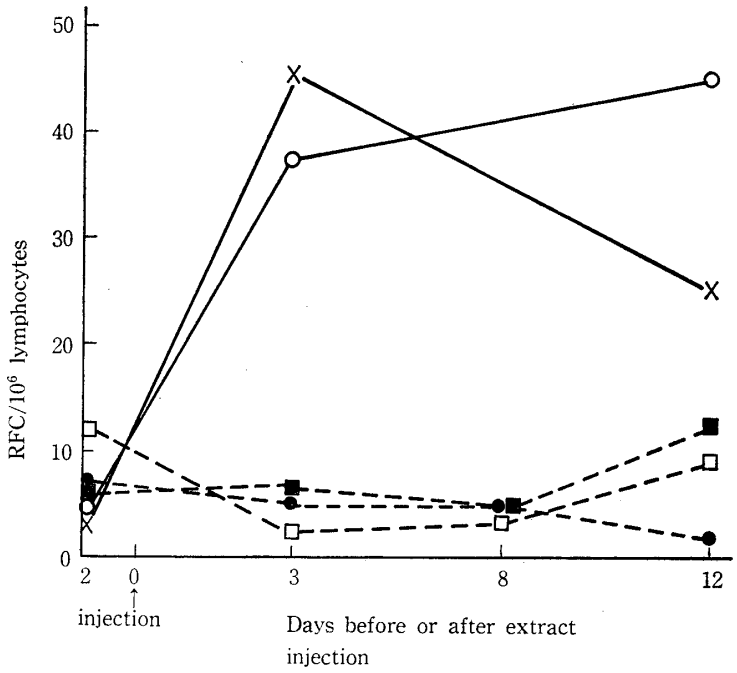

FIG. 1. Augmentation of sheep erythrocyte rosette forming cells (RFC) in blood lymphocytes of chicken receiving the leucocyte extract. The leucocytes extracts of chickens administered the immune RNA (○), the nonimmune RNA (□) or with no RNA (●) were injected. The leucocytes extracts from chickens administered immune RNA were treated with deoxyribonuclease 1 (■) or with ribonuclease A (×), and injected. Each curve represents the mean value of three chickens.

Discussion

On the maintenance of immune RNA, Kurashige *et al.* (3) reported that immune RNA was replicated in the host cells. On the mechanisms of the replication of immune RNA, Mitushashi *et al.* (8) suggested that new DNA is produced by an immune RNA-dependent DNA polymerase. Present experiments showed that a DNase sensitive substance contained in leucocyte extract from fowl that had received immune RNA augmented RFC. One possible explanation for these results may be the production of new DNA in the leucocytes which received the immune RNA. However, the DNase-sensitive substance could not be prepared from the

leucocytes of fowls which received the immune RNA only 1 or 2 times, but only from those which had received more than 3 injections (Data not shown). Therfore, the production of the DNase-sensitive substance might be triggered by a serial stimulation of the immune RNA, although the mechanisms remain unknown.

On the other hand, the present author showed (9) that the DNA of immunologically competent lymphoid cells was incorporated into the nucleus of L cells and suggested that the antibody synthesis ability in immunologically competent lymphoid cells might be transferred to the L cells if these cells had the genes for antibody synthesis.

Therefore, the results presented here may introduce an interesting and important subject, i.e. whether or not RFC by DNase sensitive substance is induced by transfer mechanisms similar to those involved in the transformation in micro-organisms. These mechanisms are now under investigation.

References

1) Fishman, M., *Nature* (London) **183**, 1200 (1959)
2) Mannick, J.A., and Egdahl, R.H., J. Clin. *Invest.* **43** 2166 (1964)
3) Kurashige, S., and Mitsuhashi, S., *J. Immunol.* **108** 1034 (1972)
4) Inooka, S., *Tohoku J. Agr. Res.*, **30**, 1 (1979)
5) Inooka, S., *Tohoku J. Agr. Res.*, **30**, 20 (1979)
6) Boyum, A., *Nature* (London) **204**, 793 (1964)
7) Dishe, Z., In "The nucleic acids" (E. Chargaff and J.N. Davidson, Eds) pp. 285–305, Academic Press, New York, (1955)
8) Mitsuhashi, S., Saito, K., and Kurashige, S., *Ann. N.Y. Acad. Sci.*, **207**, 160 (1973)
9) Inooka, S., *Tohoku J. Agr. Res.*, **17**, 279 (1967)